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13. ABSTRACT (Maximum 200 words)

Male Sprague-Dawley rats were restrained in a plastic box and positioned with the long axis of the body parallel to the electric field. Radiation was delivered at 1.2. GHz as a continuous wave (CW) with power densities of 5 and 15 mW/cm^2, or as a pulsed wave (PW) of 36% duty factor and a ratio of peak to average power density of 2.8. At the end of the exposure period rats were sacrificed by decapitation or inactivation by exposure to 5 kW for 300 msec. The hypothalamus and brain stem (H;BS) were quickly dissected and frozen in liquid nitrogen.

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The results of this study were presented at the Second Annual Meeting of the Bioelectromagnetics Society, September, 1980, San Antonio, TX and were abstracted in Bioelectromagnetics 1:236, Abst. #110, 1980.

The data have also been submitted as a journal article to Bioelectromagnetics.

The work was performed by Rex D. Stith, Ph.D. and David N. Erwin, Ph.D. and associated technical help of the Radiation Physics Branch, USAF School of Aerospace Medicine, Brooks AFB and the Department of Physiology and Biophysics, University of Oklahoma Health Sciences Center, Oklahoma City, OK.

ABSTRACT

Male Sprague-Dawley rats were restrained individually in a polyethylene box and placed in the far field of 1.2 CH_3, parallel to the electric field. Rats were irradiated for 30 minutes with either no power, continuous wave, or pulsed wave radiation of 5 or 15 mW/cm^2, average density. Groups of rats were also employed which had been subjected to stress or external heating in place of microwave radiation. At the end of the exposure period, rats were sacrificed by guillotine and hypothalamus, brain stem, and blood samples obtained. Tissues were quick-frozen and stored at $-20^{\circ}C$ until analyses. Brain tissues were assayed for tyrosine hydroxylase activity (TH) and norepinephrine (NE) and dopamine (DA) concentrations. Results reveal that hypothalamic TH and DA, but not NE were depressed after exposure to microwaves. Brainstem TH and DA were also depressed, but not to as great a degree as in hypothalamus, and mainly to 15 mW/cm^2 (CW or PW) rather than 5 mW/cm^2. Brainstem NE was unaffected. These data reveal that effects of acute microwave exposure on the brain can be detected. The changes observed were apparently not due to elevated adrenal corticoid levels on to heating of the animals. Further investigation is suggested to clarify this phenomenon and its relationship to the physiology of the organism. Keywords: Radiation effects, Exposure physiology (KT

INTRODUCTION

The last few years have seen a resurgence of research interest in achieving a quantitative understanding of the relationships between the biological effects of microwave radiation and the physical variables that cause them. Because it is known that microwave radiation at sufficiently high power levels can produce heating and associated adverse effects, the emphasis of current research is on investigating both the effects of exposures at relatively low power densities and the mechanism underlying these effects. Many reports have shown the central nervous system (CNS) to the one the systems of the body most sensitive to microwave radiation. Alterations in bioelectric function, metabolism, neuroendocrine status, and behavior have been reported as resulting from exposure to microwave radiation. However, most of the studies reported to date have utilized power density levels generally considered to be thermogenic. In the studies reported herein, we investigated the acute effects of exposure to 1.2 GHz microwave radiation at power densities of 5 mW/cm^2 and 15 mW/cm^2, continuous (CW) and pulsed-wave(PW).

This study was based upon the hypothesis that if microwave radiation results in an alteration in the physiological state of the animal that it may be mediated by the CNS. Furthermore, any effect of microwaves on the CNS that results in altered hormonal status, behavior, or other control functions of the brain, may be reflected in changes in tyrosine hydroxylase activity and/or norepinephrine or dopamine concentrations. We chose this neurotransmitter system as a model because many of the fiber pathways are known, it is involved in activation-stress reactions, a large amount of literature is available with respect to the biochemistry and physiology of this system, and sensitive biochemical assays are available to accurately and reproducibly measure changes that may occur in the system.

MATERIALS AND METHODS

Male Sprague-Dawley rate were purchased from Timco Laboratory, Houston, Tx. Rats were housed in the veterinary animal facility of the School of Aerospace Medicine, Brooks AFB, TX. A standard light/dark cycle with food and water ad libitum were employed.

The microwave exposure facilities of the Radiation Physics Branch of the Radiation Biology Division, USAF School of Aerospace Medicine were used in this study. The rats were restrained in a clear plastic box, placed in an anechoic chamber with temperature and humidity controlled, and positioned in the far field of the horn such that the long axis of the body was parallel to the electric field. Radiation was generated by a Cober model 1831 microwave generator and transmitted to an American Electronics Laboratory model H5001 horn by a flexible cable. Power density measurements were made with a National Bureau of Standards EDM-1B probe. Specific absorption rates (SAR) were calculated from calorimetric data obtained from irradiated rat cadavers by the method of Allen and Hurt (1978).

Microwave radiation was delivered at 1.2 GHz as continuous wave(CW), or as pulsed wave(PW) of 9 millisecond pulse width, 40 pulses per second, and a ratio of peak to average power output of 2.8. average power densities were 5.0 and 15 mW/cm^2.

After the 30 minute exposure period, rats were immediately sacrifice by guillotine. Blood was collected in heparinized tubes and the brain was quickly excised. Each brain was dissected to isolate the hypothalamus and brain stem. Blood samples and brain tissues were quickly frozen in liquid nitrogen and stored at $-20^{o}C$ until analysis. All samples for analyses were maintained frozen and brought by air to the University of Oklahoma Health Sciences Center, Oklahoma City, OK. All laboratory analyses were performed in the Department of Physiology and Biophysics.

Two control groups, in addition to the sham-irradiated rats, were employed. One group of rats was subjected to a combination of ether-immobilization stress for 30 minutes before sacrifice. The other group was placed in a controlled temperature-humidity oven. The temperature and humidity were varied to produce a rise in body temperature (2° C) that duplicated that which occurred upon exposure to microwaves. The duration of the elevated temperature was 30 minutes and the animals were immediately sacrificed.

Tyrosine hydroxylase activities were assayed by the method of Waymire, et al. (1979), in which ^{14}C-tyrosine was converted by the hydroxylase of the sample to ^{14}C-dopa. Hog kidney aromatic amino acid decarboxylase was added to cleave the labeled carboxyl group to form $^{14}CO_2$, which was trapped in solubilizer. Samples of trapped $^{14}CO_2$ on filter paper were counted in a liquid scintillation counter and the data converted to nanomoles of $^{14}CO_2$ evolved/mg protein/hour.

Norepinephrine and dopamine concentrations in the hypothalamus and brain stem were determined by the radioenzymatic assay described by Coyle and Henry (1973) and as modified by Cuello, et al. (1973). In this assay, an extract of the tissue sample is incubated with catechole-O-methyl transferase(COMT), ^{3}H-S-adenosyl-methionine and is converted to a labeled derivative. The metabolites, 3-methoxytyramine (3-MT) and normetanephrine (NMN) were separated by thin layer chromatography. Spots corresponding to standard 3-MT and NMN were scraped directly into liquid scinillation vials and counted. Results were expressed as picograms of norepinephrine or dopamine per mg tissue.

Plasma samples were assayed for adrenal corticoid levels by competitive protein binding (Murphy, 1967). Data were calculated as total plasma 11- oxy-corticoids in mg per 100 ml blood.

Statisticals analysis was performed by analysis of variance, Newman-Keul's sequential range test, and by student's t test. The acceptable level for significance was $p=.05$.

RESULTS

Figures 1-2 illustrate tyrosine hydroxylase (TH) activities in the hypothalamus and brain stem of the various groups of rats after exposure to microwave radiation. Neither acute ether-immobilization stress nor an external hyserthermal environment resulted in TH activities significantly different from sham-irradiated (zero power) controls in the hypothalamus (0.92 ± 0.11) and brain stem (0.27 ± 0.05). In the hypothalamus from rats sacrificed immediately after 30 minutes of microwave exposure (Figure 1), power densities of 5 and 15 mW/cm continuous wave, 15 mW/cm^2 pulsed-wave, but not 5 $mW.cm^2$ PW resulted in significantly lower enzyme levels (0.36 - 0.54) compared to sham-irradiated controls. In the brainstem (Figure 2), only exposure to 15 mW/cm^2 PW resulted in a level of TH activity (0.17 ± 0.03) lower than that of controls (0.27 ± 0.05).

It is readily apparent from Figures 3 and 4 that norepinephrine levels in the hypothalamus and brain stem were not statistically different from control values of 901 ± 167 and 418 ± 102 pg/mg tissue, respectively. However, dopamine levels were significantly depressed in both brain regions after exposure to microwaves. In the hypothalamus, all but the exposure at 15 mW/cm^2, pulsed-wave, resulted in significantly lower dopamine levels (Fig. 5). In the brain stem, exposure to 15 mW/cm^2 under both CW and PW conditions, resulted in the most dramatic depression of dopamine levels. Pulsed-wave irradiation at 5 mW/cm^2 also yielded a significantly lower dopamine level, but not to the extent as 15 mW/cm^2.

Plasma levels of adrenal corticosteroids were also measured, and the results are displayed in Tables I. Those rats that were subjected to a combined stress regimen for 30 min possessed plasma corticoid levels far above those in any of the other groups. Those rats exposed to 5 mW/cm^2, CW and PW, and those exposed to 15 mW/cm^2 PW had a mean plasma corticoid level significantly greater than control. Even though those values were elevated, they were still significantly

less than the mean value from the stressed group. In comparing the plasma corticoid data with TH, norepinephrine, and dopamine data, there are no apparent correlations between them which would indicate a cause: effect relationship between, for example, corticoid levels and dopamine values.

DISCUSSION

The data recorded from these experiments are interesting because in several cases they reveal dramatic effects due to exposure to microwave radiation, but the effects are not consistent and are difficult to correlate with power density or CW versus PW.

The data from the three control groups (Figures 1 and 2) are important because they illustrate that severe, acute stress and thermal "loading" cannot account for the changes observed in TH activities after microwave exposure. Whereas the effect of the stress paradigm was to greatly elevate total plasma 11-oxycorticoids (Table 1), those high levels do not correlate with changes in TH or noredrenergic neurotransmitter concentrations. Neither can thermal changes within the animal account for the alterations seen after microwave irradiation.

It has been adequately demonstrated that stress affects both TH activity and catecholamine levels in the brain (Diez, et al., 1977; Palkovits, et al., 1975). Foot shock, formalin injection, immobilization and cold exposure have been demonstrated to result in increased TH activity in whole brains and in discrete hypothalamic nuclei. The results of acute microwave exposure, however, were depressed levels of TH. The stressors employed in this experiment did not elevate TH activities, presumably because their duration was only 30 minutes. In most studies reported in the literature, the stressors were applied for 2-4 hours. Stresses of various kinds result in a fall in norepinephrine and dopamine content of the hypothalamus (Diez, et al., 1977; Palkovits, et al., 1975). Whereas we detected no change in norepinephrine content, there were significant declines in dopamine levels after microwave radiation.

It is possible that in the very short time course of exposure employed in this study, the TH activity levels were depressed due to microwave irradiation and that change represents the primary effect of the exposures. The decline in dopamine levels reflect the lower activities of TH. The effect of depressed TH levels on norepinephrine may not have had time to be expressed in the short time-course employed.

This investigation reveals that more work is necessary to elucidate the relationships between microwave radiation and the adrenergic nerve system of the brain at enzymatic and neurotransmitter levels, the time course of the interaction, reversibility of effects, dependency of effects on power density and mode of irradiation (CW or PW). Further studies are in preparation in which rigid controls for the effects of stress, various power densities and durations of exposure will be employed to further clarify the effects of exposure to microwave on unrestrained animals.

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FIGURE 1: TYROSINE HYDROXYLASE ACTIVITY IN RAT HYPOTHALAMUS IMMEDIATELY AFTER EXPOSURE TO MICROWAVE IRRADIATION

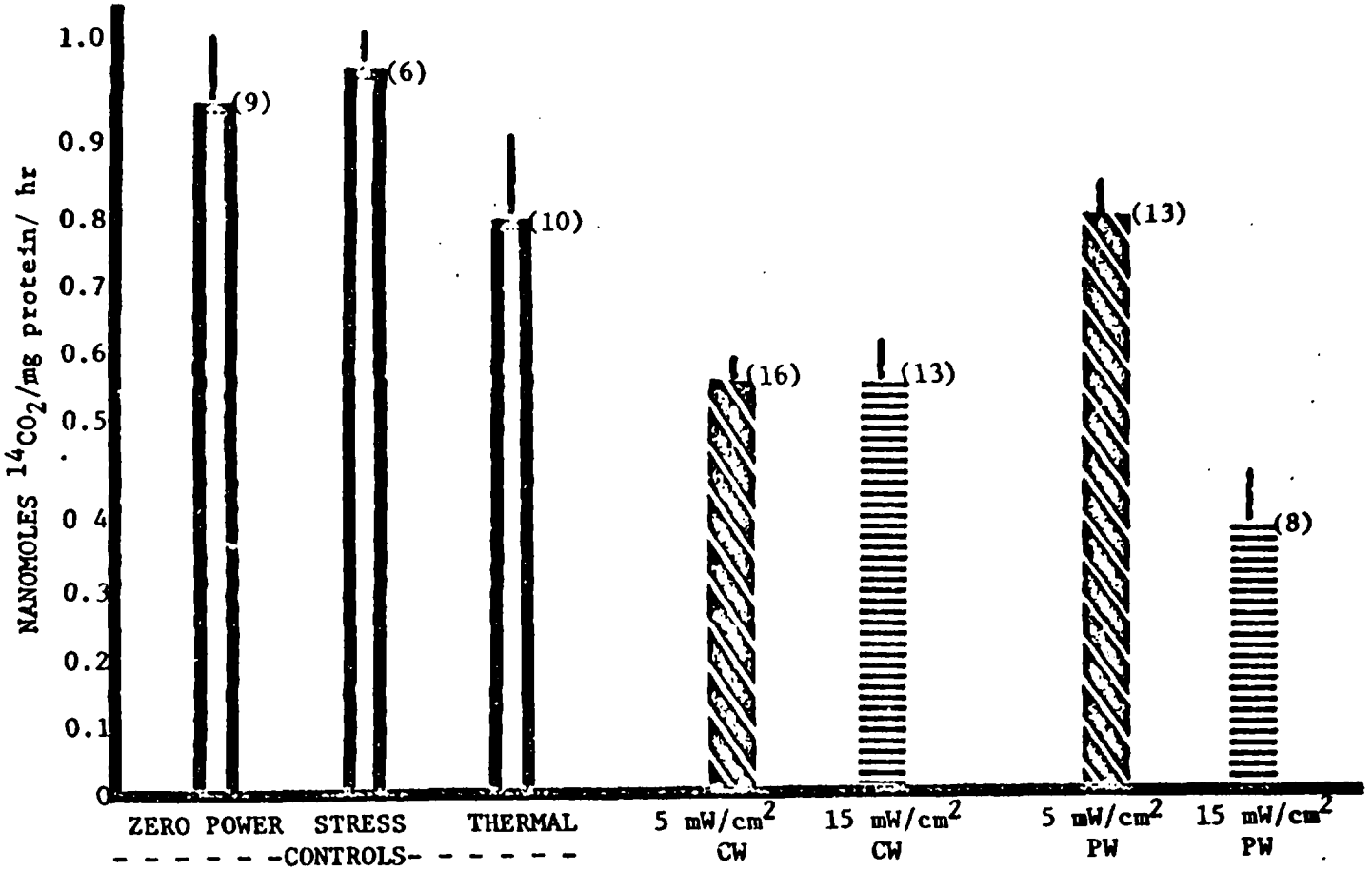

FIGURE 2: TYROSINE HYDROXYLASE ACTIVITY IN RAT BRAIN STEM IMMEDIATELY AFTER EXPOSURE TO MICROWAVE IRRADIATION

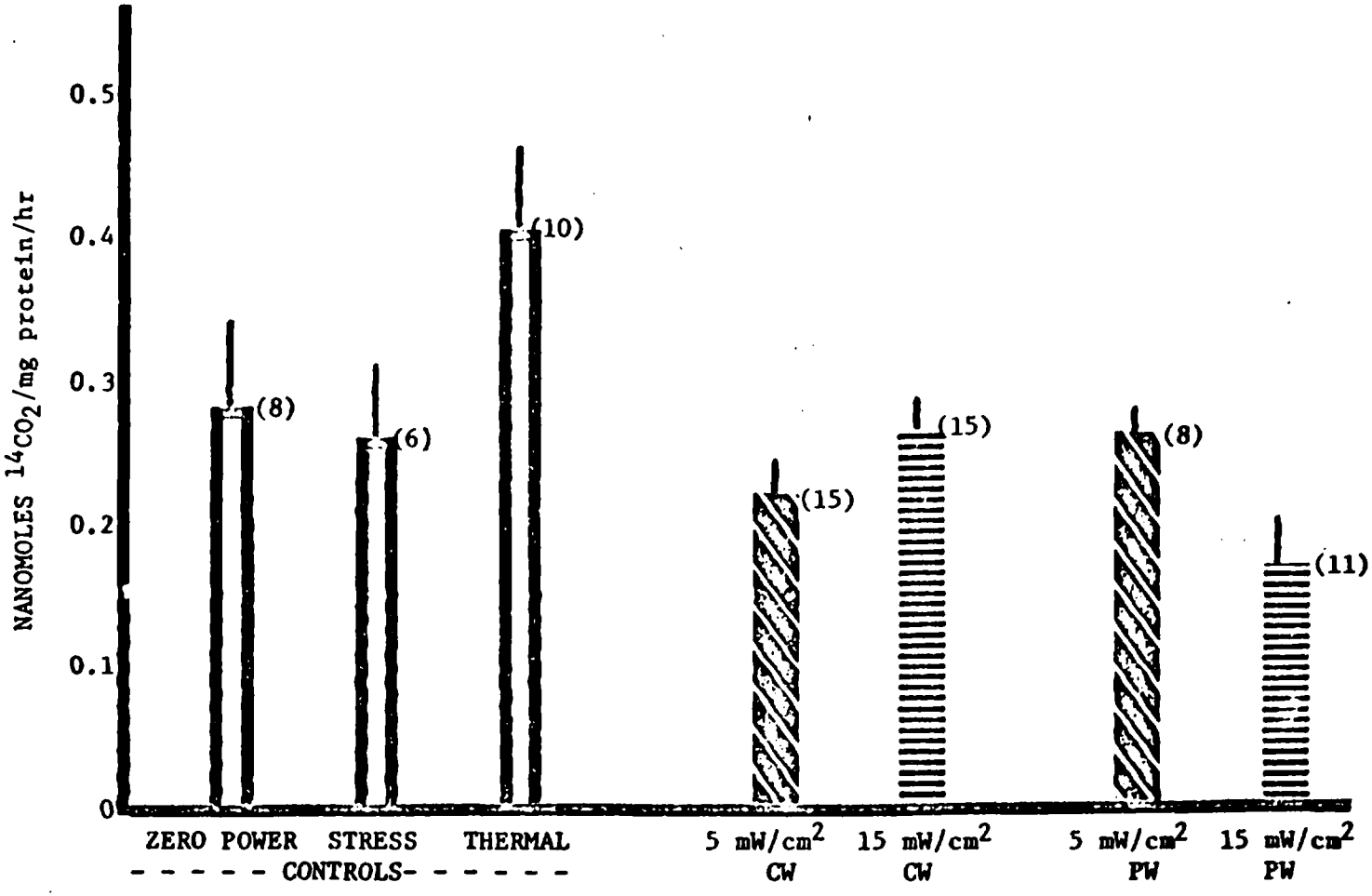

FIGURE 3: NOREPINEPHRINE CONTENT OF RAT HYPOTHALAMUS IMMEDIATELY AFTER EXPOSURE TO MICROWAVE IRRADIATION

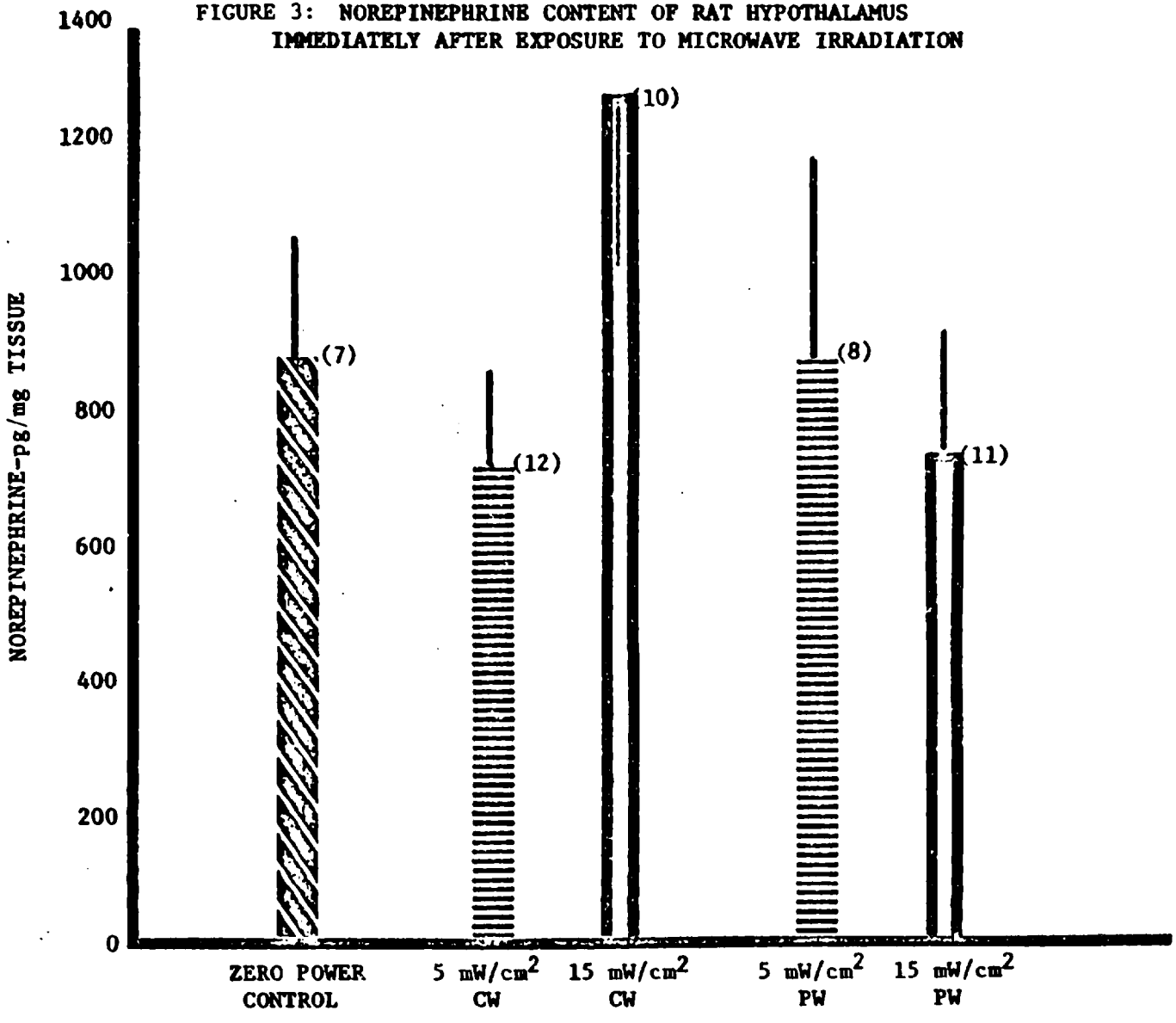

FIGURE 4: NOREPINEPHRINE CONTENT OF RAT BRAIN STEM
IMMEDIATELY AFTER EXPOSURE TO MICROWAVE IRRADIATION

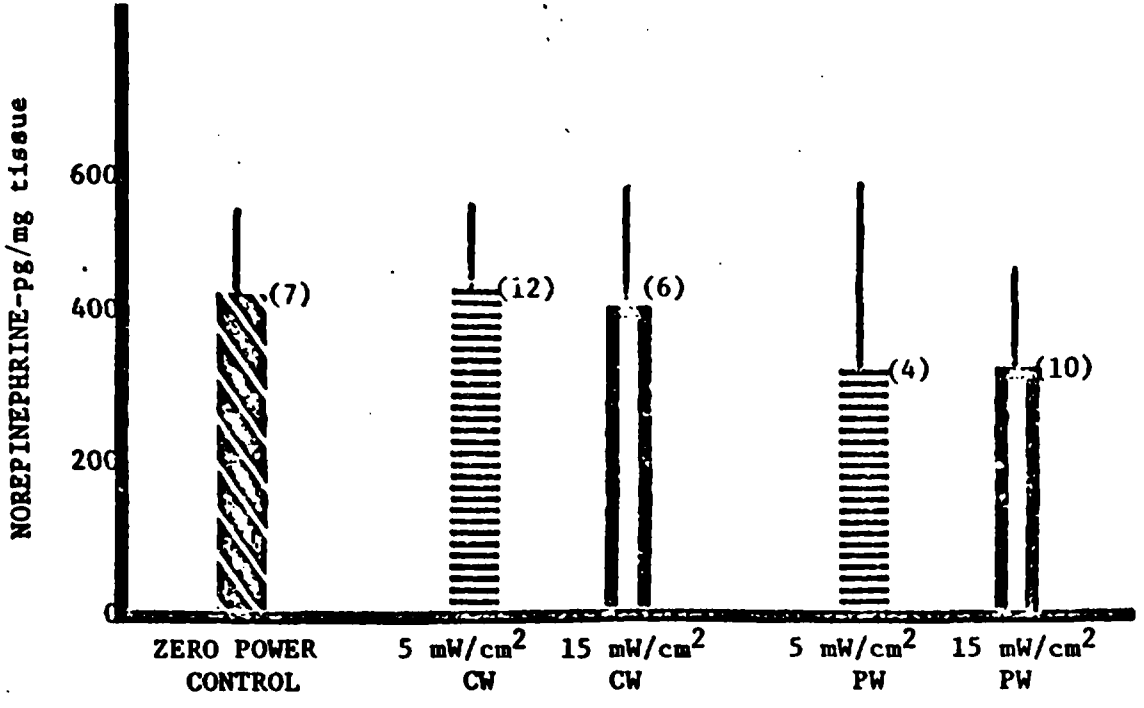

FIGURE 5: DOPAMINE CONTENT OF RAT HYPOTHALAMUS IMMEDIATELY AFTER EXPOSURE TO MICROWAVE IRRADIATION

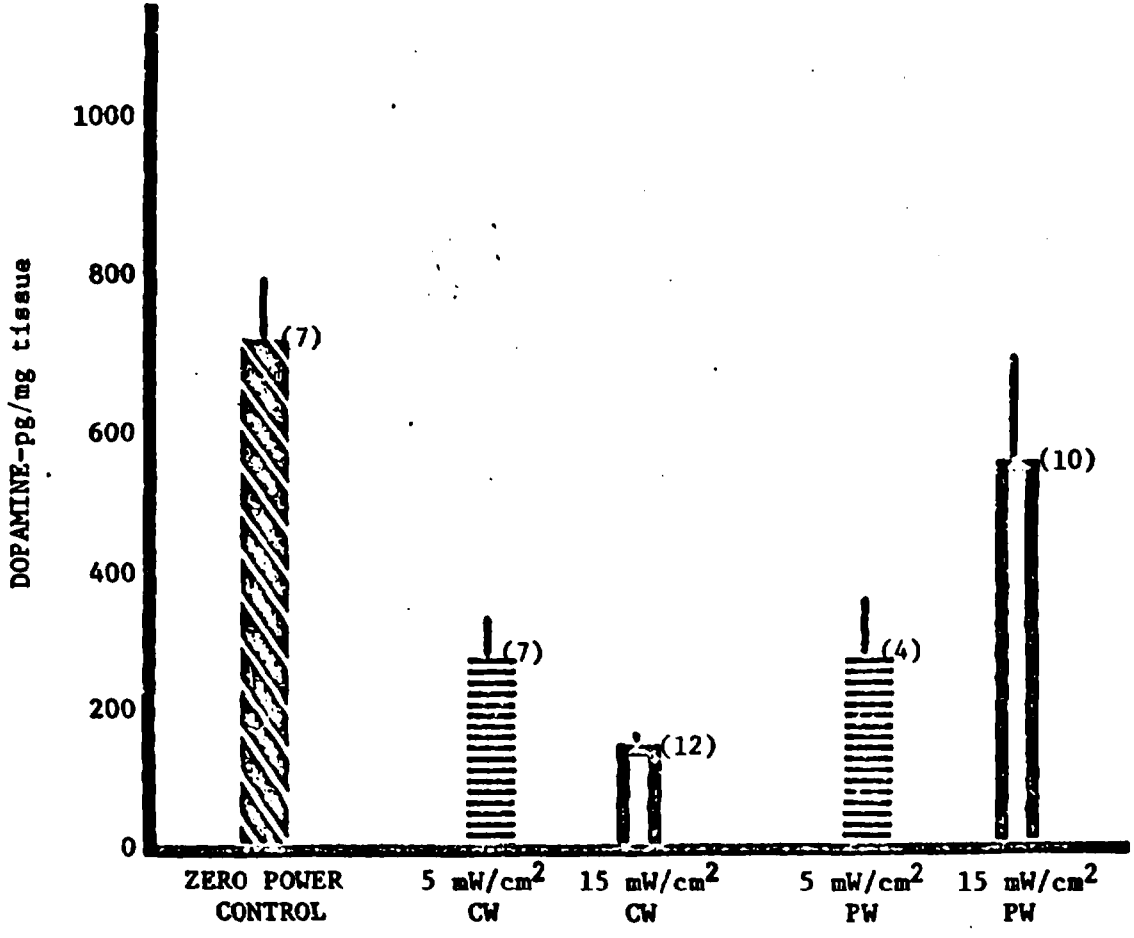

FIGURE 6: DOPAMINE CONTENT OF RAT BRAIN STEM
IMMEDIATELY AFTER EXPOSURE TO MICROWAVE IRRADIATION

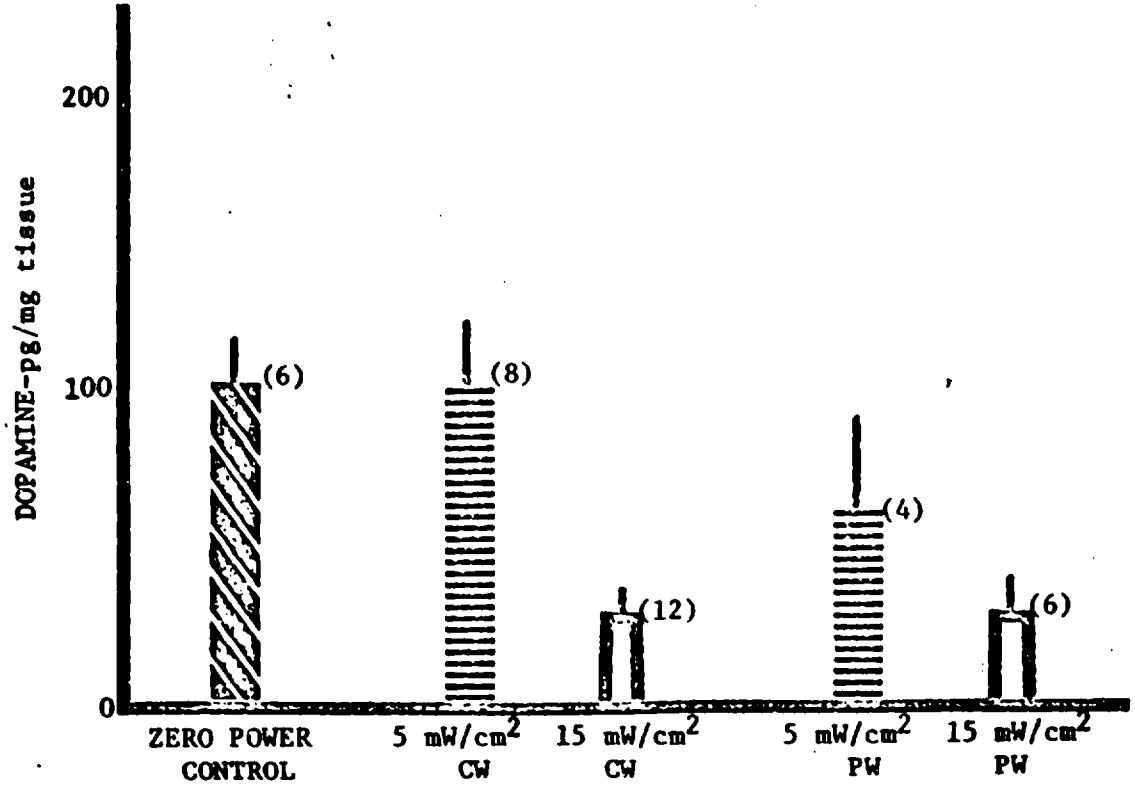

TABLE I.

PLASMA 11-OXYCORTICOIDS (μg/100 ml) FROM SHAM-IRRADIATED, STRESSED, AND MICROWAVE IRRADIATED RATS

Sham-Irradiated	Stressed	mW/cm^2 5 CW	5 PW	15 CW	15 PW
25.8±3.9	59.8±1.1	39.7±3.3	43.7±3.3	25.4±4.5	36.9±3.0
% of Control	232	154	169	98	143

Values are mean ± S.E.M. of 10 rats per group.

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